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14. ABSTRACT
In order to test the phenomenon of JP8 induced hormesis and mutagenesis in Drosophila, several experiments were performed using samples over 5000 individuals in each experiment. The JP8 doses were progressively decreased from 5 ul ti 0.5 ul in 1000 ml of air. Exposures were for 12 hours and survival was counted after 10 days. At a dose of 5 ul, the survival was 80% which increased (or lethality decreased) at lower doses. Below this dose, there was no significant decrease in the survival. Still lower doses need to be tested to investigate if there is a hormetic dose at lower levels. Mutagenecity experiments did demonstrate that the JP8 is mutagenic in all germ cell stages of Drosophila.

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JP8 INDUCED MUTAGENESIS AND HORMESIS

STATEMENT OF OBJECTIVES

The goal of the proposed work is to complement the ongoing research on JP8 genotoxicity and undertake preliminary studies on JP8 induced hormesis. Following objectives are proposed.

1. To determine the survival rates of Drosophila by exposing them to various concentrations of gaseous JP8 for different periods of time. A survival curve will established for the use in the following experiments.

2. To determine the rates of sex linked recessive lethal mutations for four dose points and obtain a dose response. These dose response profiles will be generated for the four germ cell types, i.e., for spermatogonia, spermatocytes, spermatids and the spermatozoa.

3. To determine the possibility of hormesis induced by low doses of JP8. This phenomenon has been demonstrated in cell cultures exposed to chemicals. We will test this phenomenon at organismic level using survival and/or life span as the end point. It is possible that the stress caused by JP8 at animal level may induce an adaptive response for the survival of adult males and /or females of Drosophila.

The report contains results of the experiments performed the first two objectives. A few of these experiments may be repeated this year. The third objective will be investigated in the coming year as described in the report. Preliminary experiments for this objective are already being initiated. These will form a basis for extensive experiments.

The Compound

500 ml of JP8 was obtained from Dr. Peter Robinson of Wright-Patterson Air Force Base. The fuel was filtered and stored in refrigeration for further use.

Drosophila strains

As a wild type strain, we used a Canton-S strain maintained in our laboratory for the last twenty years. We have voluminous data on the spontaneous sex-linked recessive lethal mutation rate in this strain. This rate, 0.065% has been obtained from a sample of approximately 80,000 tests. For sex-linked recessive lethal tests bar eye (M_5) strain was used. We have performed over a million tests using this strain during the past several years and find that it is quite suitable for the purpose.

The experimental Drosophila stocks were maintained in growth chambers maintained at 25°C and 60% R.H. For regular stock maintenance, and for overnight virgin collection, the cultures will be maintained at 18°C and 60% R.H.

Treatment

Inhalation

This consists of drawing a known volume of saturated vapor of the compound into a hypodermic syringe. This volume is then injected through a septum into a 1000-ml jar containing the individuals to be treated. A magnetic stirrer mixes the vapor with the air in the container. After the required period of exposure the container is capped with a wire mesh lid

and the gaseous chemical is blown out using pressurized air. The concentration of the compound in ppm by volume can be easily calculated using the vapor pressure (p) of the compound in the formula, $C = 10^2 \times p/760$. We have been using this method for the last several years (Kale and Baum 1979).

50 ml of JP8 was kept in a 1000-ml jar and was allowed to evaporate and saturate the air in jar. Using a 500-ml syringe, different amounts of this saturated vapor was drawn (through a septum) and this was injected in another 1000-ml jar with Drosophila males. After the exposure period, males were transferred to regular medium. Number of living or dead was counted the next day. These survival data are given in Table 1. None of these exposures provided a LD50 (or similar) dose point. Therefore in the next set of experiments, different amounts of liquid JP8 was added on a filter paper in a jar with Drosophila males. These data are given in Table 2. These experiments started with doses as high as 1000μL of liquid JP8. The amount of liquid was gradually lowered 100 fold to 10μL. The survival data showed large variations due to unknown reasons. Mutation experiments were performed at the does of 10μL, 20μL, and 30μL of liquid evaporates in 1000μL of air. Mutation data are given in Tables 3, 4, and 5.

The conventional (F_2) sex-linked recessive lethal test

Treated males were serialized (numbered) and mated individually to 10

virgins in bottles for a period of two days to obtain the first 2-day brood. It has been shown that a male can mate as many as 5 or more virgins in one single day and, therefore, this number of 10 virgins per brood is required for proper sectioning of the germ line and to avoid mixing of the germ cell types (Kale 1971). After this first 2-day mating period (first brood), the males were separated and supplied with a second set of 10 virgins to obtain a second 2-day brood. In case of males treated as adults only, six such 2-day broods were cultured. This brooding procedure has been used by us for the last seven years and allows us to sample spermatozoa in the first brood, spermatids in broods 2 and 3; spermatocytes in broods 4 and 5 and spermatogonia in later broods (Kale and Baum 1979).

The delayed (F_3) sex-linked recessive lethal test.

Of the several negative F_2 cultures available from each treated male, 10 to 20 cultures were chosen and from each of these, 10-15 Red Bar virgins were collected. These were pair mated to Bw males to obtain F_3 cultures. These were scored and a lethal, if any, was a delayed lethal, the rest being negative F_3 cultures.

Results

The results of experiments performed to test the survival of different doses of gaseous JP8 are given in Tables 1 and 2. These results were used to determine the doses at which mutation experiments were undertaken. Results of the induced mutation frequencies at different doses are given in Tables 3 to 5.

Table 1: Survival of Drosophila Males Induced by Various Concentrations of Saturated Vapor of Filtered JP8.

Amount of Vapor	No. of Males Treated	Age of Males	Exposure Period	Survival	
				Live	Dead
100ml	100	24-36	18 hrs	0	All
200ml	100	24-36	18 hrs	0	All
300ml	100	24-36	18 hrs	0	All
400ml	100	24-36	18 hrs	0	All
0ml	40	24-36 hrs	0 hrs	40	0
100ml	40	24-36 hrs	6 hrs	34	4
200ml	40	24-36 hrs	6 hrs	30	10
*300ml	40	24-36 hrs	6 hrs	32	8
*400ml	40	24-36 hrs	6 hrs	31	8
* Perhaps vapor in 1000ml jar was not saturated.					
500ml	50	24 hrs	10 hrs	23	14
600ml	50	24 hrs	10 hrs	20	20

Table 2: Survival of 24-36 Hours Old Drosophila Males Induced by Various Concentrations of Gaseous JP8*.

Amount of JPG Liquid in 1000 ml Jar	No. of Males Treated	Age of Males	Exposure Period	Survival	
				Live	Dead
50µl	100	24-36 hrs	6 hrs	none	All
100µl	100	24-36 hrs	6 hrs	none	All
150µl	100	24-36 hrs	6 hrs	none	All
200µl	100	24-36 hrs	6 hrs	none	All
1000µl	100	24-36 hrs	6 hrs	none	All
10µl	50	24-36 hrs	4 hrs	41	9
20µl	50	24-36 hrs	4 hrs	15	35
30µl	50	24-36 hrs	4 hrs	3	47
40µl	50	24-36 hrs	4 hrs	3	47
Experiment:					
JP8-1					
30µl	175	24-36 hrs	4 hrs	48	127
JP8-2					
20µl	110	24-36 hrs	4 hrs	70	40
JP8-3					
10µl	50	24-36 hrs	4 hrs	40	10

Table 3: Induction of Sex Linked Recessive Lethal Mutation by Low Doses of Gaseous JP8. (10µl in 1000ml of air)

10µl

Brood #	Germ Cell	No. of Chromosomes Tested	No. of Mutations	% Mutation
I.	spermatozoa	2002	11	0.55
II.				
III.	spermatids	973	15	1.54
IV.	spermatocytes	721	6	0.83
V.				
VI.	spermatogonia	515	4	0.78

Table 4: Induction of Sex Linked Recessive Lethal Mutation by Low Doses of Gaseous JP8. (20µl in 1000ml of air)

20µl

Brood #	Germ Cell	No. of Chromosomes Tested	No. of Mutations	% Mutation
I.	spermatozoa	2394	11	0.45
II.				
III.	spermatids	677	7	1.03
IV.	spermatocytes	1241	10	0.80
V.				
VI.	spermatogonia	1547	12	0.77

Table 5: Induction of Sex Linked Recessive Lethal Mutation by Low Doses of Gaseous JP8. (30µl in 1000ml of air)

30µl

Brood #	Germ Cell	No. of Chromosomes Tested	No. of Mutations	% Mutation
I.	spermatozoa	1162	8	0.69
II.				
III.	spermatids	1126	1	0.08
IV.	spermatocytes	1939	0	0.0
V.				
VI.	spermatogonia	1358	5	0.36

As can be seen from the table 1, saturated vapor experiments gave variable results. This can be attributed to the dilutions of dose when larger amount of vapor such as 400 ml and above are drawn from the 1000ml container. Tables 1 and 2. Therefore in the second set of experiments liquid JP8 was allowed to evaporate in the 1000 ml jar in which Drosophila males were already present. In these experiments, longer exposures, such as 6 hours killed all males. Four hour exposures however did not kill all the males and doses were undertaken at three dose points of 10, 20, and 30 µl of JP8.

The results of the mutagenesis experiments are given in tables 3, 4, and 5. These data were compared with the historical spontaneous mutation rate in our wild type (C-S) stock which we have been using for the last 25 years. The control rate of sex linked recessive lethal mutations in this strain is 1 in 1000 or 0.01%. We used this control rate to compare the observed mutation frequencies induced by the three doses in four types of germ cell stages. Except of one exception of one germ cell stage (Table 5, spermatocytes), the gaseous JP8 induced significantly higher numbers of mutations in all stages in all three dose points. Therefore JP8 is definitely mutagenic in Drosophila. The germ cell sensitivity analysis shows that spermatids are more sensitive. This aggress very well with other similar experiments with other chemicals.

In addition to the survival and mutagenesis experiments, we also performed a preliminary experiment to study a possible hormetic effect of JP8 on survival of Drosophila. The samples were however quite small and we were not able to observe a hormetic effect. However, these samples were very small and doses are not well identified. We plan to repeat these preliminary experiments and initiate other experiments to study the hormesis if any in the coming year.